REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*: 30/09/2003
2. REPORT TYPE:
3. DATES COVERED: 01/07/1997 - 30/09/2000

4. TITLE AND SUBTITLE: Biogeochemical factors affecting microbial transformation of co-occurring contaminants in sediments from a saltmarsh Superfund site

5a. CONTRACT NUMBER: 97-NCERQA-10
5b. GRANT NUMBER: N0014-97-1-0955
5c. PROGRAM ELEMENT NUMBER:
5d. PROJECT NUMBER:
5e. TASK NUMBER:
5f. WORK UNIT NUMBER:

6. AUTHOR(S): Frischer, Marc, E.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Skidaway Institute of Oceanography
10 Ocean Science Circle
Savannah, GA 31411

8. PERFORMING ORGANIZATION REPORT NUMBER:

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

10. SPONSOR/MONITOR'S ACRONYM(S): ONR

11. SPONSOR/MONITOR'S REPORT NUMBER(S):

12. DISTRIBUTION/AILABILITY STATEMENT
Distribution Unlimited

20031010 037

13. SUPPLEMENTARY NOTES

14. ABSTRACT A common difficulty associated with devising bioremediation strategies for many contaminated sites is the co-occurrence of several classes of toxic chemicals. In the case where mixed contaminants are present, the likelihood of interactions between candidate transformation pathways is high and greatly complicates remediation processes. Because these pathways are believed to involve complex microbial consortia, this program seeks to investigate the linkages among biogeochemical parameters, microbial activity, microbial diversity and community structure, as they apply to the transformation of co-occurring contaminants. It is hypothesized that a fundamental understanding of these linkages will lead to the development of enhanced *in situ* remediation strategies.

15. SUBJECT TERMS
Contaminated Sediments
Marine
Bioremediation
Polychlorinated Biphenyls
Mercury
Bacteria

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
Unclass	Unclass	Unclass	Unlimited		Marc E. Frischer
					19b. TELEPHONE NUMBER *(Include area code)* 912/598-2308

FINAL REPORT

GRANT #: N00014-97-1-0955

PRINCIPAL INVESTIGATOR: Dr. Marc E. Frischer

INSTITUTION: Skidaway Institute of Oceanography

GRANT TITLE: Biogeochemical factors affecting microbial transformation of co-occurring contaminants in sediments from a saltmarsh Superfund site.

AWARD PERIOD: 01 JUL 1997 – 30 SEP 2000

OBJECTIVE: To identify linkages between contaminant chemistry, biogeochmistry, and microbial activity/diversity in contaminated saltmarsh sediments and to experimentally evaluate strategies for remediating saltmarsh sediments contaminated with co-occurring organic contaminants (PCBs) and heavy metals (mercury).

APPROACH: Via multidisciplinary collaborations a series of laboratory scale anaerobic incubations with contaminated sediments were initiated and maintained under both methanogenic and sulfidogenic conditions. The effect on contaminant transformation, primarily of PCBs in the form of weathered Aroclor 1268, in the presence of mercury were evaluated under these conditions. Additionally, the importance of pH, temperature, addition of biphenyl primers, and simple nutrient additions were evaluated. Concurrent with these studies the effect on the activity of microbial communities and the structure of microbial communities were analyzed to identify the key processes and organisms responsible for contaminant transformation.

ACCOMPLISHMENTS: Evidence of *in situ* dechlorination of Aroclor 1268 (Avg = 8.5 chlorines per biphenyl) was documented in PCB/Hg contaminated sediment from the LCP chemical site. These findings suggested dehalogenation under native sulfate sulfidogenic conditions present in the saltmarsh environment. Because elevated methyl mercury porewater concentrations were coincident with the presence of slightly dechlorinated PCBs, the association between sulfate reduction and dehalogenation activity was further strengthened. Therefore, one of the primary objectives of this project was to investigate the linkage between sulfate reduction and PCB dechlorination. Controlled microcosm studies were initiated to quantitatively link sulfate reduction with PCB dechlorination. These studies indicated that some dechlorination activity could be induced under both methanogenic and sulfidogenic conditions in the presence of high mercury concentrations, but only with the addition of a PCB "primer". After 3-months of incubation, the sulfidogenic treatment primed with 23456-pentachlorobiphenyl (a "meta" primer) exhibited the greatest decrease in Aroclor 1268. In addition, the distribution of lower chlorinated PCBs, presumed to be dechlorination products, differed among the treatments and primer choice, suggesting that different microbial dechlorination activities were inducible. However, the extent of dechlorination was low in each case. Studies to determine the effect of nutrient addition and nutrient starvation were conducted. In these

studies nutrient addition (complex and defined) and nutrient starvation did not enhance PCB dechlorination.

To identify potentially important microbial consortia involved in contaminant transformation, studies were undertaken to associate the structure of microbial communities associated with pristine and contaminated sediments. Characterization of microbial community structure was determined using several molecular approaches including: direct sequencing of the 16S rRNA gene, cell blot hybridization with suites of phylogenetic broad group-specific 16S rRNA targeted oligonucleotide probes and, Amplified Ribosomal DNA Restriction Analysis (ARDRA). Microbial community composition was examined seasonally from sediment cores collected along a contaminant gradient at the LCP Superfund site and at uncontaminated sites in conjunction with measurements of microbial activity, biogeochemical parameters, and contaminant distribution. Characterization of 16S rDNA clone collections by ARDRA and sequence analysis suggests that microbial diversity is at least as high in contaminated sediments as those found in other environments. Although sulfate reducing bacteria (SRB) accounted for the majority of activity and probe hybridization signal, respectively, the delta proteobacteria did not numerically dominate the microbial consortium as determined by sequence analysis of clones obtained from pristine or contaminated sites. Within the SRB populations, the abundance of *Desulfobacterium* was positively correlated to the presence of contaminants (Hg & PCB). Processes other than the presence of contaminants could explain the abundance and distribution differences of other groups of SRBs. These studies suggest the importance of diverse microbial assemblages for the tolerance and transformation of complex contaminant mixtures in saltmarsh sediments and highlight specific consortia that may be involved in the remediation of PCB and mercury in saltmarsh sediments.

CONCLUSIONS: Natural assemblages of saltmarsh bacteria appear to be capable of surviving and transforming heavily chlorinated PCBs in situ. Members of the Beta Proteobacteria may be involved in this process. Specific stimulation of sulfate reduction or methanogenic activity did not enhance bioremediation of either mercury or PCBs in these saltmarsh sediments. Similarly, nutrient starvation did not enhance contaminant transformation. These results suggest that a general increase in bacterial activity or forcing nutrient limitation will not promote the natural attenuation of PCBs in contaminated saltmarsh sediments.

SIGNIFICANCE: These studies demonstrate that natural saltmarsh bacterial communities that are present in mercury and PCB contaminated sediments have the potential to transform (remediate) these contaminants. Our studies also suggest that a general manipulation of two major bacterial processes, sulfate reduction and methanogensis, do not result in significant enhancement of contaminant transformation. Our findings suggest that specific bacteria, perhaps in the Beta Proteobacteria group, are responsible for PCB dechlorination.

STUDENTS:

Leena, D. Palekar, M.S. Microbiology, University of Georgia, Athens, August 2002. Thesis Title: Anaerobic Microbial Dechlorination of Polychlorinated Biphenyls.

Theresa Fu, M.S. Environmental Engineering, Georgia Institute of Technology, Atlanta Georgia, December 2001. Thesis Title: The Influence of Sulfate-Reducing Bacteria and Spartina Alterniflora in Simulated Salt Marsh Systems.

Michele A. Newton Healy, M.S. Environmental Engineering, Georgia Institute of Technology, Atlanta Georgia, December 1999. Thesis Title: Community Structure and Population Dynamics of Sulfate-Reducing Bacteria in an Equilibrating Saltmarsh Mesocosm by 16S Ribosomal RNA Sequence Comparisons.

PUBLICATIONS:

Palekar L.D., Maruya K.A., Kostka J.E., Wiegel J. (In Press). Dehalogenation of 2,6-dibromobiphenyl and 2,3,4,5,6-pentachlorobiphenyl in contaminated estuarine sediment. *Chemosphere.*

King, J.K., J.E. Kostka, M.E. Frischer, F.M. Sanders, and R. Jahnke. (2001). A quantitative relationship that demonstrates mercury methylation rates in marine sediments are based on the community composition and activity of sulfate-reducing bacteria. *Environ. Sci. and Tech.* 35:2491-2496.

Frischer, M.E., J.M. Danforth, M.A. Newton Healy, and F. M. Saunders. (2000). Whole cell versus total RNA extraction for the analysis of microbial community structure using 16S rRNA targeted oligonucleotide probes in saltmarsh sediments. 66:3037-3043. *Appl. Environ. Microbiol*

King, J.K., J.E. Kostka, M.E. Frischer, and F.M. Saunders. (2000). Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments. 66:2430-2437. *Appl. Environ. Microbiol.*

Reyes, N.S., M.E. Frischer, And P.A. Sobecky. (1999). Characterization of mercury resistance mechanisms in marine sediment microbial communities. *FEMS Microbial Ecology. 30:273-284.*

PRESENTATIONS:

Palekar L.D., Maruya K.A., Kostka J.E., and J. Wiegel. Dechlorination patterns of PCBs by anaerobic consortia of estuarine sediment microorganisms, ASM National Conference, May 2000, Los Angeles, CA.

Reddy, C.M., Eglinton, T.I., Heraty, L.J., Holt, B.D., Sturchio, N.C., Lake, J.L. and K.A. Maruya. Stable chlorine isotope analysis of chlorinated organic contaminants. SETAC 20th Ann. Mtg., Philadelphia, PA, Nov.14-18, 1999.

Maruya, K.A., R.F. Lee, M.E. Frischer, J.E. Kostka, and H.L. Windom. 1998. Bioavailability and bioremediation of co-occurring PCBs and mercury in saltmarsh sediments. Society of Environmental Toxicology and Chemistry 19th Annual Meeting. Charlotte, NC. November 15-19, 1998.

Kostka, J.E., L.B. Cowden, J. King, and M.E. Frischer. 1999. Microbial biogeochemistry of saltmarsh sediments co-contaminated with mercury and PCBs. American Society for Microbiology General Meeting, 1999, Chicago, IL.

J.K. King, M.E. Frischer, J.E. Kostka, and F.M. Saunders. Identification of sulfate-reducing, phylogenetic groups most capable of methylating mercury in pure culture and organic acid amended sediment slurries. American Society for Microbiology General Meeting, 1999, Chicago, IL.

Frischer, M.E., J.D. Danforth, L.B. Cowden, and J.E. Kostka. 1999. Characterization of microbial assemblages associated with saltmarsh sediments co-contaminated with mercury and PCB. American Society for Microbiology General Meeting, 1999, Chicago, IL.

N.S. Reyes, M.E. Frischer, And P.A. Sobecky. 1999. Molecular characterization of marine microbial communities from PCB and Hg-contaminated saltmarsh sediments. American Society for Microbiology General Meeting, 1999, Chicago, IL.

J.E. Kostka, F.M. Saunders, J.K. King, and M.E. Frischer. (1999) Characterization of the role of macrophytes and microbial populations in governing the fate/ transport of methyl mercury in highly contaminated estuarine sediments. 1999 Conference on Hazardous Waste Research: Gateways to Environmental Solutions, Sponsored by EPA-HSRC, St. Louis, MO.

Maruya, K., R. Lee, M. Frischer, J. Kostka and H. Windom "Bioavailability and bioremediation of saltmarsh sediments contaminated with Aroclor 1268", First International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 18-21, 1998.

Maruya, K.A., R.F. Lee, M.E. Frischer, J.E. Kostka, H.L. Windom, J.K. King, and F.M. Saunders. 1999. Biotransformation of co-occurring PCBs and mercury by sulfate reducing bacteria in estuarine sediments. Society of Enviromental Toxicology and chemistry 20th Annual Meeting. Philadelphia, PA. November 14-18, 1999.

J. E. Kostka. (1997) Rates and pathways of microbial respiration in sediments of the South Atlantic, Annual Meeting of the American Society for Microbiology, Miami, FL